APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1984, p. 980–983
0099-2240/84/110980-04$02.00/0

Vol. 48, No. 5

Measurement of the Surface Free Energy of Bacterial Cell Surfaces and Its Relevance for Adhesion

HENDRIK J. BUSSCHER,* ANTON H. WEERKAMP, HENNY C. VAN DER MEI, ANTOON W. J. VAN PELT, HANS P. DE JONG, AND JOOP ARENDS

Dental School, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

Received 8 February 1984/Accepted 9 May 1984

An experimental technique is described to determine contact angles on bacterial layers deposited on cellulose triacetate filters. Measurements with water, water–*n*-propanol mixtures, and α-bromonaphthalene were employed to calculate surface free energies of various oral bacteria. Differences of 30 to 40 erg cm^{-2} were obtained for four different bacterial species isolated from the human oral cavity, if the concept of dispersion and polar surface free energies is applied. The free energies obtained were used to calculate interfacial free energies of adhesion of these bacteria from saliva onto tooth surfaces. Bacterial adhesion is energetically unfavorable, if the enamel surface free energy is less than 50 erg cm^{-2}.

Adhesion of bacteria onto tooth surfaces is a prerequisite for the formation of dental plaque and the occurrence of dental caries and periodontal disease (5). Adhesion of indigenous oral bacteria to tooth surfaces is thought to involve both specific, lectin-like interactions between complementary surface components and physicochemical surface characteristics such as charge and hydrophobicity (3, 13). A thermodynamic approach offers, in principle, a powerful tool to predict bacterial adhesion to solid substrates, because on the basis of an interfacial free energy balance, neglecting electrical charge interactions, adhesion may be expected if (1):

$$\Delta F_{adh} = \gamma_{sb} - \gamma_{sl} - \gamma_{bl} < 0 \quad (1)$$

where ΔF_{adh} is the interfacial free energy of adhesion, γ_{sb} is the solid-bacterium interfacial free energy, γ_{sl} is the solid-liquid interfacial free energy, γ_{bl} is the bacterium-liquid interfacial free energy, whereas adhesion is energetically unfavorable if:

$$\Delta F_{adh} > 0 \quad (2)$$

The interfacial free energies in Eq. (1) can be calculated from various surface chemical approaches. The equation of state approach, also employed by Absolom et al. (1), is used in most microbiological studies because of its experimental and calculational ease. Another approach, which to our knowledge has not been used in microbiological research, is based on separation of the surface free energies in a dispersion, γ^d, and a polar, γ^p, component.

According to the geometric-mean equation (4, 8), the interfacial free energy between any two surfaces 1 and 2 can be expressed in its dispersion and polar components by:

$$\gamma_{12} = \gamma_1 + \gamma_2 - 2(\gamma_1^d \cdot \gamma_2^d)^{1/2} - 2(\gamma_1^p \cdot \gamma_2^p)^{1/2} \quad (3)$$

The influence of the solid substrate on the adhesion process is subsequently described by:

$$d(\Delta F_{adh}) = (\gamma_s^d)^{-1/2}[(\gamma_1^d)^{1/2} - (\gamma_b^d)^{1/2}]d\gamma_s^d + (\gamma_s^p)^{-1/2}[(\gamma_1^p)^{1/2} - (\gamma_b^p)^{1/2}]d\gamma_s^p \quad (4)$$

If the expression presented in Eq. (4) equals zero, there is no influence of the solid substrate on the adhesion process. As can be seen (Fig. 1A), this is the case for: (i) $\gamma_b^d = \gamma_1^d$ and $\gamma_b^p = \gamma_1^p$; (ii) γ_s^d = constant, whereas $\gamma_b^p = \gamma_1^p$; (iii) γ_s^p = constant, whereas $\gamma_b^d = \gamma_1^d$. For most materials, γ_s^d is about 40 erg cm^{-2} (12) (an exception being polytetrafluorethylene, with $\gamma_s^d \approx 20$ erg cm^{-2}), and Eq. (4) can be simplified to:

$$\frac{d(\Delta F_{adh})}{d\gamma_s} = (\gamma_s^p)^{-1/2}[(\gamma_1^p)^{1/2} - (\gamma_b^p)^{1/2}] \quad (5)$$

From Eq. (5) it is obvious that $d(\Delta F_{adh})/d\gamma_s < 0$ (adhesion will be energetically more favorable as γ_s increases) if $\gamma_b^p > \gamma_1^p$, whereas $d(\Delta F_{adh})/d\gamma_s > 0$ (adhesion will be less as γ_s increases) if $\gamma_b^p < \gamma_1^p$ (Fig. 1B).

This introduction demonstrates the need for an experimental method to determine the surface free energy γ_b of bacterial surfaces. The solid as well as the liquid parameters that appear in Eq. (1) can be measured by employing contact angle measurements (2, 8). Determination of surface free energies of bacterial surfaces by contact angle measurements imposes special problems because of interactions of the bacteria with the liquid droplets and problems connected with the drying of the bacterial layer. In this report, a technique is described which is suitable for measuring contact angles on bacterial substrata. Dispersion and polar components γ_b^d and γ_b^p will be calculated from contact angles measured on a variety of oral bacteria.

MATERIALS AND METHODS

Preparation of the bacterial substrata. *Streptococcus salivarius* HB, *Streptococcus sanguis* CH3, *Streptococcus mitior* T6, and *Veillonella alcalescens* V1 were isolated from human oral cavities and stored at −20°C in 7% dimethyl sulfoxide. Streptococci were grown overnight in air with 5% CO_2 at 37°C in Todd-Hewitt broth (Oxoid Ltd., London, England), and veillonellae were grown in Todd-Hewitt broth supplemented with sodium lactate (21 ml per liter of a 60% [wt/vol] syrup) overnight in an anaerobic jar in an atmosphere containing N_2, H_2, and CO_2 (85:10:5).

Cells were harvested by centrifugation, washed twice, and suspended in distilled water.

The bacterial substrata for measuring contact angles were prepared by collecting bacterial cells on a cellulose triacetate filter (pore diameter, 0.45 μm; Gelman GA-6) to a density of 10^8 cells per mm^2. To establish a constant moisture content,

* Corresponding author.

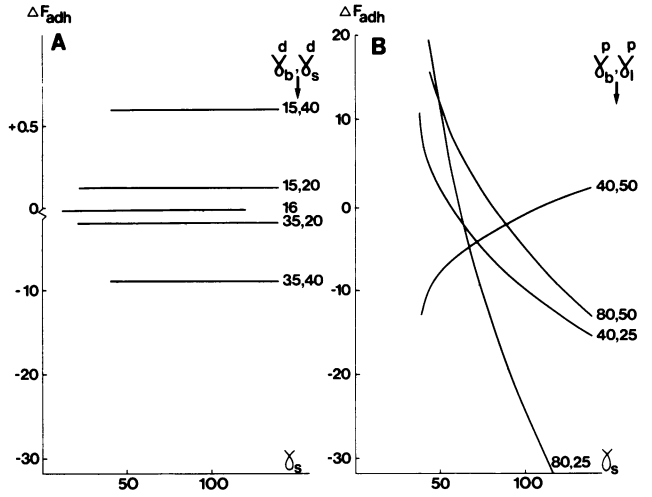

FIG. 1. Some cases for ΔF_{adh} as a function of γ_s. (A) $d(\Delta F_{adh})/d\gamma_s = 0$. In all cases $\gamma_l^d = 16$ and $\gamma_b^p = \gamma_l^p = 40$ erg cm^{-2}. The numbers denote γ_b^d and γ_s^d. Note the broken scale of the ΔF_{adh} axis. (B) $d(\Delta F_{adh})/d\gamma_s \lesseqgtr 0$. In all cases $\gamma_l^d = 25$, $\gamma_b^d = 35$, and $\gamma_s^d = 40$ erg cm^{-2}. The numbers denote γ_b^p and γ_l^p. All units are in ergs per centimeter squared.

the filters with bacteria were placed in a petri-dish on the surface of a layer of 1% (wt/vol) agar in water containing 10% (vol/vol) glycerol until the filters were mounted onto a holder. To this end, strips (width, 1 cm) were cut from the filters and fixed onto the sample disks with double-sided adhesive tape. Since subsequent dehydration of the filters because of evaporation of water was expected to influence the contact angles (1), water contact angles were measured as a function of drying time. Scanning electron microscopy was employed to determine whether the filters were completely and homogeneously covered with bacteria. The homogeneity of the deposited bacterial layer was also determined by measuring water contact angles at seven different places on a given surface.

Contact angle measurements and surface free energy calculations. Contact angle measurements by the sessile drop technique (H. J. Busscher, A. W. J. van Pelt, H. P. de Jong, and J. Arends, Colloids Surf., in press) were carried out at 25°C with water, water–*n*-propanol mixtures, and α-bromonaphthalene as wetting agents. A series of contact angle (θ) data on a given surface yields the solid surface free energy (γ_s) by fitting the data by least-squares analysis to (2):

$$\cos\theta = -1 + 2(\gamma_s^d \cdot \gamma_l^d)^{1/2} \cdot \gamma_l^{-1} + 2(\gamma_s^p \cdot \gamma_l^p)^{1/2} \cdot \gamma_l^{-1} - \pi_e \cdot \gamma_l^{-1} \quad (6)$$

in which d and p denote dispersion and polar components, respectively, of the solid γ_s and the liquid γ_l surface free energies. π_e denotes the spreading pressure, defined as the difference between the solid surface free energy γ_s against air and γ_{sv}, the solid surface free energy against a saturated vapor of the liquid employed.

As a consequence of the experimental circumstances, the saturated vapor is a mixed water–*n*-propanol vapor. Methods of calculation yielding γ_s^d, γ_s^p together with π_e, and γ_{sv} separately, were employed. Contact angles were measured 2, 4, and 6 s after the droplet was placed on the bacterial surfaces.

Liquids. All liquids used in this study and their reference states γ_l, γ_l^d, and γ_l^p have been described previously (Busscher et al., in press). The water was of high purity (10); *n*-propanol and α-bromonaphthalene were obtained from Merck AG (analytical grade).

RESULTS

A scanning electron micrograph of *S. salivarius* HB on a filter is shown in Fig. 2. The filter is completely and homogeneously covered with bacteria. The heterogeneity of the deposited bacterial layer was very small according to the water contact angle measured at seven different places on the same sample. The standard deviation of the mean was less than 2° for all bacterial species investigated.

The water contact angles, measured 2 s after the droplets were applied, are presented as a function of the drying time of the bacterial deposits (Fig. 3). It appears that reliable contact angle measurements should be carried out between 0 and 90 min after placement of the filters onto the sample disk. However, since fluctuations appeared to be more pronounced on very fresh bacterial layers, we routinely allowed a minimal 30-min drying time. Prepared bacterial deposits may be kept for several hours on the agar plates before mounting on the sample disks without affecting the contact angles.

The contact angles of all droplets on the bacterial surfaces slightly decreased as a function of time. Contact angles of water and α-bromonaphthalene droplets on one filter are presented as a function of time in Fig. 4.

To correct for this decrease, it was decided to extrapolate the data obtained at 2, 4 and 6 s linearly to 0 s. Although the data at 2, 4, and 6 s for the same bacteria on separate filters often differ markedly, linear extrapolation yielded values which were comparable within the experimental error. All contact angles obtained in this way are summarized in Table 1. The surface free energies, calculated from Eq. (6), and the

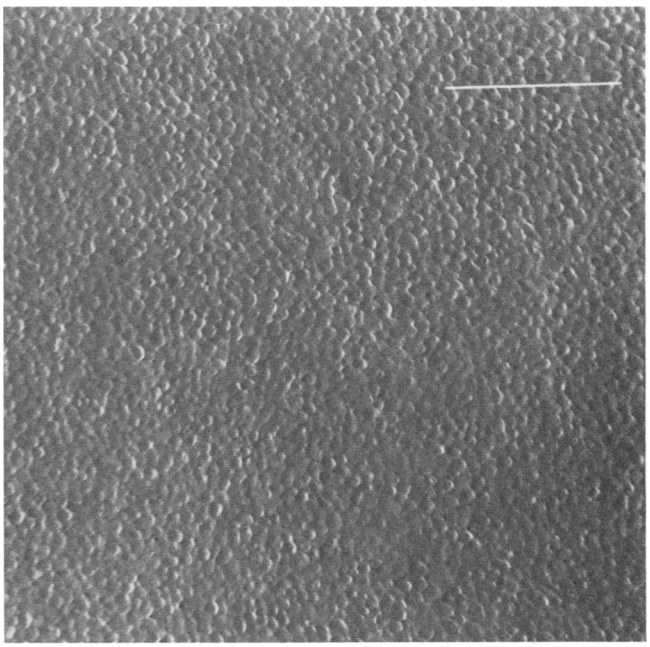

FIG. 2. Scanning electron micrograph of *S. salivarius* HB cells deposited on a cellulose triacetate filter as described in the text. Bar, 10 μm.

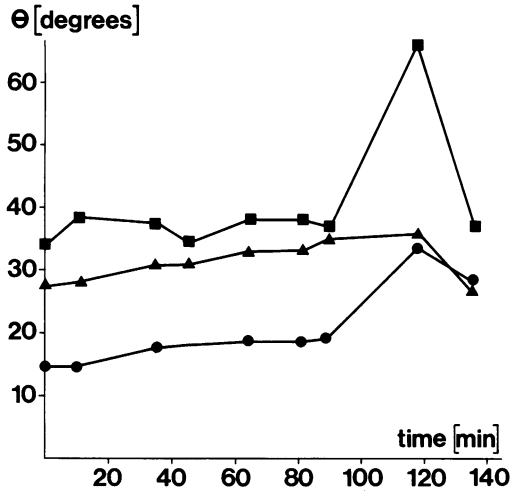

FIG. 3. Water contact angles on bacterial substrates as a function of the drying time of the substrates. The contact angles were measured 2 s after the droplet was placed on the substrates. Symbols: ●, *V. alcalescens* V1; ▲, *S. sanguis* CH3; ■, *S. salivarius* HB.

contact angles from Table 1 are presented in Table 2. It is interesting to note from the data in Table 2 that for bacterial layers, γ_{bv} is always smaller than 72 erg cm^{-2}, γ_b may be greater than 72 erg cm^{-2}, and $\gamma_b - \gamma_{bv}$ corresponds numerically with the calculated spreading pressure π_e, in accordance with the thermodynamic definition. Similar observations have been made for nonbiological materials (2).

DISCUSSION

The technique described in this report for the measurement of contact angles on bacterial deposits is easy to perform and shows good reproducibility.

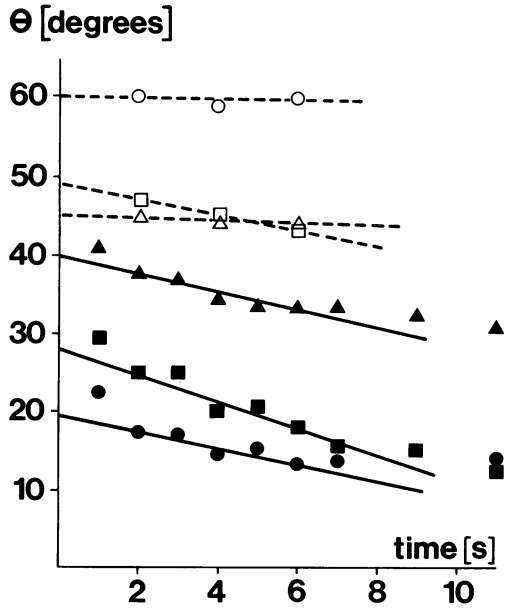

FIG. 4. Contact angles of a water droplet (solid lines) and an α-bromonaphthalene droplet (broken lines) on bacterial substrates as a function of time. Indicated are the lines through the 2-, 4-, and 6-s data points. Symbols: ●, *V. alcalescens* V1; ▲, *S. sanguis* CH3; ■, *S. salivarius* HB.

TABLE 1. Contact angles of water, water–*n*-propanol mixtures, and α-bromonaphthalene on deposits of oral bacteria

Liquid (concn [%, wt])	Contact angles (degrees) on the following bacteria[a]:			
	V. alcalescens V1	*S. sanguis* CH3	*S. salivarius* HB	*S. mitior* T6
Water	20	42	26	55
n-Propanol (0.5)	15	41	26	52
n-Propanol (1)	16	42	26	56
n-Propanol (2)	20	45	23	53
n-Propanol (3)	17	45	26	53
n-Propanol (4)	17	46	33	49
n-Propanol (8)	17	41	27	49
n-Propanol (12)	19	39	28	45
n-Propanol (17)	14	38	31	35
n-Propanol (20)	15	37	29	41
n-Propanol (40)	7	36	27	0
α-Bromonaphthalene	57	41	44	31

[a] Contact angles were corrected for a slight time-dependent decrease by linear extrapolation to 0 s.

The variation in the surface free energies γ_b and γ_{bv} obtained for the various oral bacteria is large compared with the results of Absolom et al. (1), who calculated surface free energies γ_{bv} between 66 and 70 erg cm^{-2} for five different gram-negative and gram-positive bacteria deposited on agar. Apart from using different bacteria, Absolom et al. used a different theoretical concept and employed other methods of calculation to derive surface free energies. By our method, the reduction of the solid surface free energy by vapor adsorption from the liquid droplets is taken into account, which excludes the specific influence of the liquids used on the derived surface free energies. In addition, it is possible in this way to determine solid surface free energies γ_b higher than the surface free energy of the test liquids employed (12). Thus, as can be seen from our data, two of the bacterial strains investigated have surface free energies γ_b which are significantly higher than the surface free energy of water.

A shortcoming of the equation of state approach is the discrepancy between surface free energy values derived from contact angles with different liquids. Calculation of γ_{bv} from our α-bromonaphthalene contact angles using the equation of state resulted in values ranging from 27 to 38 erg cm^{-2}, which is much lower than the values calculated from our water contact angles which ranged from 47 to 65 erg cm^{-2}.

The interaction of oral bacteria suspended in saliva with human enamel is of great importance in dentistry. In Fig. 5 ΔF_{adh}, calculated from Eq. (1), is given as a function of γ_s (γ_s^d = 40 erg cm^{-2}) for the adhesion of the oral bacteria investigated from saliva (γ_l^d = 29; γ_l^p = 24 erg cm^{-2} [6]). The adhesional energy ΔF_{adh} is positive for three of the four bacterial strains investigated at a solid surface free energy less than 62 erg cm^{-2}. Since bare, polished as well as

TABLE 2. Surface free energies of oral bacteria[a]

Bacteria	γ_{bv}	γ_b^d	γ_b^p	γ_b	π_e
V. alcalescens V1	60 ± 1	27 ± 4	74 ± 1	101 ± 4	42 ± 3
S. sanguis CH3	45 ± 1	34 ± 2	52 ± 2	86 ± 1	44 ± 1
S. salivarius HB	58 ± 2	33 ± 2	72 ± 3	105 ± 5	49 ± 2
S. mitior T6	33 ± 2	38 ± 1	30 ± 6	69 ± 6	33 ± 5

[a] Values are in ergs per centimeter squared ± the standard deviation (n = 4).

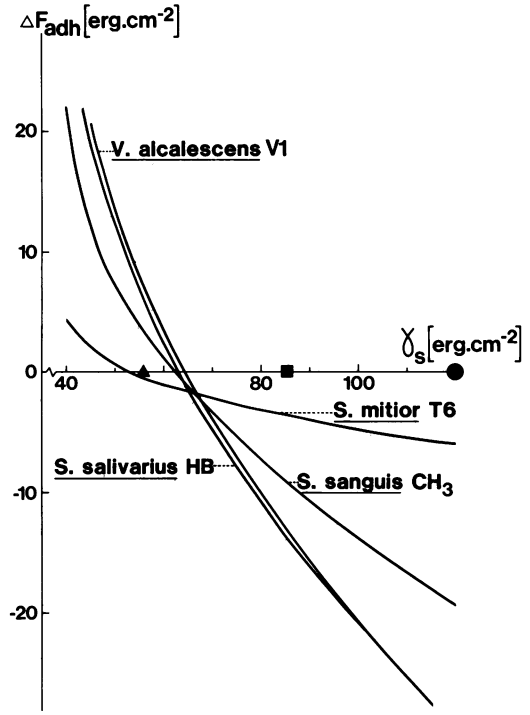

FIG. 5. ΔF_{adh} as a function of γ_s for adhesion of oral bacteria from saliva ($\gamma_l^d = 29$; $\gamma_l^p = 24$ erg cm^{-2} [6]). Along the γ_s-axis, the surface free energy data of human enamel are indicated. Symbols: ■, bare, polished human enamel (11); ▲, bare, polished human enamel after an aminfluoride treatment (2b); ●, bare, polished human enamel after salivary protein adsorption (pellicle formation) (2a).

pellicle-coated enamel have higher surface free energies (2a, 11), adhesion of bacteria would be thermodynamically favorable. Treatment of enamel with surfactants such as aminofluorides (2b) reduces the enamel surface free energy low enough to discourage adhesion of bacteria.

In most reports, bacterial adhesion is interpreted as the number of bacteria that adhere per unit area, although physics suggests that it is better to interpret bacterial adhesion in relation with ΔF_{adh} to the binding force, or the energy required to remove the adhered bacteria. (Such experiments would admittedly be very difficult.)

Some authors (1) have found very convincing linear relationships between the number of bacteria that adhere and the surface free energy, although less straightforward relations have also been found (9, 12). Whether the surface free energy γ_s is related via ΔF_{adh} to the number of bacteria adhered or to the binding force, a high, positive value of ΔF_{adh} is favorable for reducing bacterial adhesion. On the basis of these results, a drastic reduction in bacterial adhesion to, or to be more specific, the energy required to remove bacteria from, enamel surfaces can be expected if the enamel surface free energy can be permanently decreased to below about 50 erg cm^{-2}.

Conclusions. (i) The described technique is suitable for reproducible and accurate determination of contact angles on bacterial surfaces and provides a basis for estimating bacterial surface free energies. (ii) The surface free energy γ_b of oral bacteria, calculated from the dispersion and polar components concept, varies from 69 erg cm^{-2} for *S. mitior* T6 to 105 erg cm^{-2} for *S. salivarius* HB. (iii) The adhesional interfacial free energy ΔF_{adh} for the adhesion of oral bacteria on tooth enamel surfaces from saliva becomes positive if the enamel surface free energy is less than 50 erg cm^{-2}.

ACKNOWLEDGMENTS

We thank W. L. Jongebloed of the Centre for Medical Electron Microscopy for valuable assistance and Wilma Dijkstra for preparing this manuscript.

LITERATURE CITED

1. **Absolom, D. R., F. V. Lamberti, Z. Policova, W. Zingg, C. J. van Oss, and A. W. Neumann.** 1983. Surface thermodynamics of bacterial adhesion. Appl. Env. Microbiol. **46**:90–97.
2. **Busscher, H. J., A. W. J. van Pelt, H. P. de Jong, and J. Arends.** 1983. Effect of spreading pressure on surface free energy determinations by means of contact angle measurements. J. Colloid Interface Sci. **95**:23–27.

2a. **de Jong, H. P., P. de Boer, H. J. Busscher, A. W. J. van Pelt, and J. Arends.** 1984. Surface free energy changes of human enamel during pellicle formation—an in vivo study. Caries Res. **18**:408–415.

2b. **de Jong, H. P., A. W. J. van Pelt, H. J. Busscher, and J. Arends.** 1984. The effect of topical fluoride applications on the surface free energy of human enamel—an in vitro study. J. Dent. Res. **63**:635–641.

3. **Doyle, R. J., W. E. Nesbitt, and K. G. Taylor.** 1982. On the mechanism of adherence of *Streptococcus sanguis* to hydroxyapatite. FEMS Microbiol. Lett. **15**:1–5.
4. **Fowkes, F. M.** 1964. Attractive forces at interfaces. Ind. Eng. Chem. **12**:40–53.
5. **Gibbons, R. J., and J. van Houte.** 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. **29**:19–44.
6. **Glantz, P. O.** 1970. The surface tension of saliva. Odontol. Revy **21**:119–127.
7. **Neumann, A. W., D. R. Absolom, D. W. Francis, and C. J. van Oss.** 1980. Conversion tables of contact angles to surface tension. Sep. Purif. Methods **9**:69–163.
8. **Owens, D. K., and R. C. Wendt.** 1969. Estimation of the surface free energy of polymers. J. Appl. Polym. Sci. **13**:1741–1747.
9. **Pringle, J. H., and M. Fletcher.** 1983. Influence of substratum wettability on attachment of fresh water bacteria to solid surfaces. Appl. Env. Microbiol. **45**:811–817.
10. **Sampson, R.** 1977. High purity water for atomic adsorption analyses. Am. Lab. March, 1977.
11. **van Pelt, A. W. J., H. P. de Jong, H. J. Busscher, and J. Arends.** 1983. Dispersion and polar surface free energies of human enamel. J. Biomed. Mat. Res. **17**:637–641.
12. **van Pelt, A. W. J., H. J. Busscher, M. H. W. J. C. Uyen, A. H. Weerkamp, H. P. de Jong, and J. Arends.** 1984. Adhesion of *S. sanguis* to polymers over a broad range of surface free energies—an in vitro study, p. 167–177. *In* J. M. ten Cate, S. A. Leach, and J. Arends (ed.), Bacterial adhesion and preventive dentistry. IRL Press Ltd., London.
13. **Weerkamp, A. H., H. C. van der Mei, D. P. E. Engelen, and C. E. A. de Windt.** 1984. Adhesion receptors (adhesins) of oral streptococci, p. 85–97. *In* J. M. ten Cate, S. A. Leach, and J. Arends (ed.), Bacterial adhesion and preventive dentistry. IRL Press Ltd., London.